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Chemical and dimensional characterization of the renal countercurrent system in mice

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Chemical and dimensional characterization of the renal countercurrent system in mice. This study sought to identify the mechanisms leading to vasopressin (ADH)-resistant urinary concentrating defects in several genotypes of mice by analyzing the chemical composition as well as the dimensions of their countercurrent systems. As an aid to the analysis, a method was developed for estimating solute concentrations at the papillary tip by extrapolating from measurements of papillary solute concentration. The results indicate that the mechanisms causing decreased urinary concentration vary with the genotypes. In some, decreased water permeability appears to be the primary defect, leading secondarily to diminished cortico-papillary gradients for sodium and urea. In other genotypes a foreshortened countercurrent system and osmotic diuresis per nephron diminish the corticopapillary urea gradient. And in still others, a combination of these causes appears to be involved. The study shows again that various diuretic states selectively wash out the medullary urea, probably because they curtail the medullary source of urea but not that of sodium.

Characteres chimiques et importance du systeme de contre-courant renal chez la souris. Le but de cette étude était d'identifier les mécanismes responsables du défaut de concentration des urines résistant à la vasopressine (ADH) observé dans plusieurs génotypes de souris. Le moyen utilisé a été l'analyse de la composition chimique et des dimensions de leurs systèmes à contre-courant. Pour facilites cette analyse, une méthode a été élaborée qui perment calculer, pas extrapolation, la concentration à la pointe de la papille des substances dissoutes dont les concentrations ont été mesurées dans la papille. Les résultats montrent que les mécanismes responsables de la diminution de concentration urinaire diffèrent avec les génotypes. Dans certains cas, la diminution de perméabilité à l'eau semble être le défaut primaire, responsable d'une diminution secondaire des gradients cortico-papillaires du sodium et de l'urée. Dans d'autres cas, un raccourcissement du système de contre-courant et une diurèse osmotique par nephron diminuent le gradient cortico-papillaire de l'urée. Enfin dans d'autres cas ces différents mécanismes paraissent combinés. L'étude montre à nouveau que différents états diurétiques evacuent d'une façon sélective l'urée médullaire, sans doute en tarissant la source medullaire d'urée, mais non alle du sodium.

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Previous studies on mice with vasopressin (ADH)-resistant deficiencies in urinary concentration have revealed a number of different mechanisms which may be responsible for the defects [1–5]. In some genotypes (VII Os/+ and DI Os/+) renal failure, osmotic diuresis per nephron, and a foreshortened countercurrent system may be the major factors; in the DI +/+ Non-Severe and DI +/+ Severe genotypes, it may be varying degrees of reduced water permeability of the distal nephron; and in at least one, DI Os/+, foreshortened short loops of Henle appear to contribute to the defect [4].

It was the purpose of the present study to assess the possible role of these mechanisms in each of the genotypes by analyzing the chemical composition as well as the dimensions of their countercurrent systems.

A severe deficiency in ADH-induced water permeability can be easily detected through chemical analysis of papillary tissue. In such cases [6] the hypotonic final urine will have a significantly lower osmolality than any portion of the hypertonic medulla or papilla. However, when the defect in water permeability is mild and the lack of osmotic equilibration between collecting duct fluid and papillary interstitium may therefore be small, knowledge of the osmolality at the very tip of the papilla is important. Most methods [7] used for determining solute concentrations in renal tissues yield an underestimation of the value at the very tip of the papilla. Several mm of the papilla must be removed in order to get enough tissue for analysis, and a rise of several hundred mOsm/kg H_2O can occur within a single mm, depending on the slope of the cortico-papillary solute gradient.

Although micropuncture techniques might overcome this difficulty in some instances, they too have limitations. Sampling of collecting duct fluid and vasa recta blood at precisely the same horizontal level is essential in assessing osmotic equilibration. More importantly, except for the Sakai technique [8] which in itself somewhat compromises papillary function, the papilla is inaccessible to micropuncture in most adult species. This limitation made micropuncture unsuitable for the present investigation since certain of the severe urinary concentrating defects become manifest only in older animals. We have therefore developed a method for estimating the solute concentration at the papillary tip by extrapolation of the measured tissue value. Although this method yields no more than a rough estimate of the tip value, applying the correction is nevertheless deemed worthwhile because it permits a more complete analysis than unextrapolated tissue values alone.

Methods

Five groups of adult mice were tested (Table 1). Four abnormal genotypes, VII Os/+, DI Os/+, DI +/+ Non-Severe and DI +/+ Severe, and a control group, VII +/+, were used. Urine osmolality in DI +/+ mice may range from about 2,000 mOsm/kg H_2O to less than 100 mOsm/kg H_2O; this variation occurs in part as a function of age and in part because of genetic variation (Valtin, H.: unpublished observations). In an effort to clarify the responsible abnormalities in the DI +/+ genotype, animals were chosen on the basis of whether they excreted urine of a fairly high or a very low osmolality; so-called DI +/+ Non-Severe mice had a mean age of 14 weeks and a mean urine osmolality of 1,406 mOsm/kg H_2O; in DI +/+ Severe animals, these values were 54 weeks and 149 mOsm/kg H_2O, respectively.

All animals were kept in an air-conditioned room maintained at 21° to 25°C. They had free access to water and food (Purina Labena pellets, Ralston Purina Co., St. Louis, Mo.).

Tissue analyses. Mice were induced to urinate on a clean, dry surface; the samples were stored briefly in capillary tubes between columns of water-equilibrated mineral oil and then placed in the sample holder of a nanoliter osmometer (Clifton Technical Physics, New York, N.Y.). The animals were anesthetized with ether and the kidneys were removed and processed for determination of sodium, potassium, and urea by methods previously described [6]. Corresponding tissues from both kidneys of the same animal (i.e., papilla, medulla, and cortex) were pooled, weighed to the nearest 0.1 mg, and placed in a tissue grinder containing about 0.8 ml of cold, doubly distilled water measured accurately by weighing it to the nearest 0.1 mg. In the case of DI Os/+ animals, it was sometimes necessary to pool samples from three kidneys in order to obtain enough tissue for analysis. Sodium and potassium were measured by flame photometry (Instrumentation Laboratory, Inc., Lexington, Mass.) and urea by a colorimetric method [9].

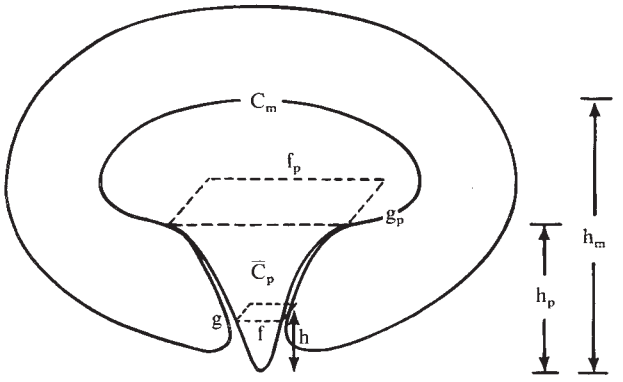
Total tissue solute concentrations were calculated as urea + 2(Na+K), and expressed as mmoles/kg of wet tissue. The papillary values were converted to mmoles/kg tissue water, assuming an increasing water content of papillary tissue with increasing severity of diuresis [6, 10, 11]. The following water contents were assumed (Table 3): VII +/+, 80%; VII Os/+, 82%; DI +/+ Non-Severe, 85%; DI +/+ Severe, 90%; DI Os/+, 87%. Cortical values were converted to mmoles/kg tissue water, assuming that 75% of cortical tissue is water in all groups.

Dimensional analysis. The data summarized in Table 2 were obtained on a separate series of mice identical to those listed in Table 1. Animals used for dimensional analysis were stunned by a blow to the head and then killed by breaking their necks. Both kidneys were cut eccentrically along a longitudinal plane, in the same manner as those used in the earlier series for chemical measurements. The cut surface of the larger portion, with the medulla and papilla exposed, was then measured using a dissecting microscope (Wild M 5 stereo-microscope) equipped with an adjustable eyepiece micrometer (Oknor, Leitz, Wetzlar, Germany) which was calibrated with a 2 mm stage micrometer. The distance from cortico-medullary junction to papillary tip, in the long axis of the papilla, was measured. The papilla was removed and weighed to the nearest 0.1 mg (Model H 10, Mettler Instrument Corporation, Princeton, N.J.), and its length was determined. Both kidneys of each mouse were processed in this manner. In order to utilize the dimensional data obtained on one series of mice for estimating papillary tip concentrations from the chemical analyses on another series (see below), the dimensions were corrected for the slightly greater size (i.e., weight) of papillary samples in the latter group.

Calculation of solute concentrations at the papillary tip. The following calculations were performed to estimate the tissue concentration of sodium, urea, and total solutes [i.e., urea + 2(Na+K)] at the very tip of

the papilla. The respective values for papillary solutes (Table 1) were converted from mmoles/kg wet tissue to mmoles/kg tissue water, assuming the papillary tissue water content listed in Table 3, and extrapolating the mean concentration for each group using the linear dimensions given in Table 2.

The method, summarized in Fig. 1, involves two major assumptions: 1) that the shape of the average papillary sample approximates a pyramid of height, h_p, with a rectangular base of width, f_p, and depth, g_p; and 2) that the gradient for sodium, urea, and total solutes from cortico-medullary junction to the papillary tip is linear [10, 12, 13] and that it can therefore be described by the equation



$$C_{tip} = C_m + \frac{1}{1 - {}^3/_4 \cdot h_p/h_m} \cdot (\bar{C}_p - C_m)$$

Fig. 1. *Diagram illustrating the method for estimating solute concentrations at the papillary tip through tissue analysis.* All terms are defined in the text. The equation, which was used to estimate papillary tip values in this study, is the algebraic simplification of the integrations.

$$C = C_m + b \cdot (h_m - h) \tag{1}$$

where C = the concentration of the solute in question at a point h mm from the papillary tip;
C_m = the concentration of that particular solute at the cortico-medullary junction;
h = distance in mm from the papillary tip, of any point along the long axis of the papilla;
h_m = distance from papillary tip to cortico-medullary junction;
b = slope of the straight line describing the relationship between C and $(h_m - h)$.

The total volume of the papillary sample, V_p, is given by

$$V_p = \int_0^{h_p} f \cdot g \cdot dh \tag{2}$$

where f and g, the width and depth of the pyramid at height h, are given by

$$f = f_p \cdot h/h_p, \tag{3}$$

$$g = g_p \cdot h/h_p. \tag{4}$$

The papillary content of the solute in question, S_p, is given by

$$S_p = \int_0^{h_p} f \cdot g \cdot C \cdot dh. \tag{5}$$

Substituting for C from equation (1), this may be divided into two parts:

$$S_p = \int_0^{h_p} f \cdot g \cdot C_m \cdot dh + \int_0^{h_p} f \cdot g \cdot b \cdot (h_m - h) \cdot dh$$

$\therefore$ substituting V_p from equation (2) and rearranging,

$$S_p = C_m \cdot V_p + b \cdot \int_0^{h_p} f \cdot g \cdot (h_m - h) \cdot dh. \tag{6}$$

The mean papillary concentration of the particular solute, $\bar{C}_p$, is given by

$$\bar{C}_p = S_p/V_p.$$

Substituting for S_p from equation (6) and for V_p from equation (2),

$$\bar{C}_p = C_m + b \cdot \int_0^{h_p} f \cdot g \cdot (h_m - h) \cdot dh \Big/ \int_0^{h_p} f \cdot g \cdot dh$$

$$\therefore b = (\bar{C}_p - C_m) \cdot I \tag{7}$$

where

$$I = \int_0^{h_p} f \cdot g \cdot dh \Big/ \int_0^{h_p} f \cdot g \cdot (h_m - h) \cdot dh. \tag{8}$$

The concentration of the solute at the papillary tip is given from equation (1) as

$$C_{tip} = C_m + b \cdot h_m.$$

Substituting for b from equation (7), we have

$$C_{tip} = C_m + (\bar{C}_p - C_m) \cdot I \cdot h_m. \tag{9}$$

$I \cdot h_m$ can be analytically evaluated by carrying out the integrations in equation (8) after substituting for f and g from equations (3) and (4). Algebraic simplification then leads to the result that

$$I \cdot h_m = \frac{1}{1 - {}^3/_4 \cdot h_p/h_m}, \tag{10}$$

$$\therefore C_{tip} = C_m + \frac{1}{1 - {}^3/_4 \cdot h_p/h_m} \cdot (\bar{C}_p - C_m). \tag{11}$$

This provides a simple formula for extrapolation of the measured solute concentration, $\bar{C}_p$, to the esti-

mated value at the papillary tip, C_{tip}. The "correction factor" $\frac{1}{1 - 3/4 \cdot h_p/h_m}$ requires measurement only of h_p, the length of the papillary sample which is analyzed, and of h_m, the distance from the papillary tip to the cortico-medullary junction. Furthermore, this correction factor can be applied to papillae of any pyramidal shape, not just to those with a rectangular base.

The correction factor was derived for each group of mice, using the values for h_p and h_m listed in Table 2. The cortical values listed in Table 1 were converted to mmoles/kg tissue water, assuming that 75% of cortical tissue is water [6, 10, 11]. These converted values were used to represent the concentrations at the cortico-medullary junction, C_m. The tip concentrations and cortico-papillary gradients (b) in Table 3 were then calculated with the use of the correction factor for each group.

Results were analyzed statistically using the computer programs of Dartmouth College (Kiewit Computation Center). Standard errors of the extrapolated tip values were calculated on the assumption that the standard errors of all experimental measurements were mutually independent.

Table 1. Analyses of urine and renal tissues in control mice and mice with ADH-resistant urinary concentrating defects

Type		No. of Mice ♀	No. of Mice ♂	Urine osmolality *mOsm/kg H_2O*	Papilla *mmoles/kg WT*[a]	Medulla *mmoles/kg WT*	Cortex *mmoles/kg WT*
VII +/+		6	6	2,764±232[b]			
(Control)	Na				368±21.6	147± 9.5	78± 3.6
	K				79± 3.4	73± 1.6	84± 1.3
	Urea				508±41.7	129±14.7	26± 2.0
	Sum[c]				1,403±83.9	571±31.6	350± 8.8
VII Os/+		7	6	2,141±138[d]			
	Na				321±21.8	121± 4.5[d]	85± 4.4
	K				71± 4.7	72± 2.0	83± 1.6
	Urea				280±37.7[d]	92± 6.8[d]	36± 4.6
	Sum				1,062±74.0[d]	479±10.5[d]	370±10.4
DI +/+		6	5	1,406±77[d]			
Non-Severe	Na				264±15.3[d]	118± 4.8[d]	61± 1.8[d]
	K				66± 2.0[d]	75± 1.8	86± 1.7
	Urea				227±17.4[d]	63± 2.7[d]	9± 0.6[d]
	Sum				884±44.0[d]	449± 6.9[d]	304± 3.8[d]
DI +/+		3	2	149± 19[d, e]			
Severe	Na				214±12.1[d]	108±11.0[d]	69± 2.8[e]
	K				40± 4.3[d, e]	70± 2.0	82± 2.8
	Urea				38±14.8[d, e]	19± 9.6[d, e]	4± 2.4[d, e]
	Sum				546±43.6[d, e]	374±29.1[d, e]	307± 1.8[d]
DI Os/+		6	5	222± 18[d, e]			
	Na				197±11.0[d, e]	95± 3.2[d, e]	73± 2.0[e]
	K				75± 4.5	73± 1.9	80± 2.5
	Urea				56± 7.5[d, e]	30± 2.8[d, e]	16± 1.1[d, e]
	Sum				599±31.0[d, e]	367± 9.6[d, e]	321± 7.0[d, e]

[a] *WT* = wet tissue.
[b] Mean ± SEM.
[c] Sum = Urea + 2(Na + K).
[d] Significantly different when compared to VII +/+ mice ($P < 0.05$).
[e] Significantly different when compared to DI +/+ Non-Severe mice ($P < 0.05$).

Table 2. Dimensional data on the countercurrent system of control mice and mice with ADH-resistant urinary concentrating defects

Type	No. of mice		h_m [a]	h_p [a]	Sample weight [a]
	♀	♂	*mm*	*mm*	*mg*
VII +/+ (Control)	3	3	5.41 ±0.199 [b]	3.16 ±0.209	2.18 ±0.105
VII Os/+	3	3	4.11 ±0.330 [c]	2.70 ±0.310	2.13 ±0.075
DI +/+ Non-Severe	3	3	4.91 ±0.170	2.95 ±0.142	1.98 ±0.091
DI +/+ Severe	1	2	4.99 ±0.256	2.65 ±0.260	2.14 ±0.114
DI Os/+	3	3	4.25 ±0.135 [c, d]	2.60 ±0.205	1.62 ±0.142 [c]

[a] h_m = distance from papillary tip to cortico-medullary junction; h_p = calculated length of papillary samples which were analyzed chemically; sample weight = weight of chemically analyzed papillary sample.
[b] Mean ± SEM.
[c] Significantly different when compared to VII +/+ mice ($P<0.05$).
[d] Significantly different when compared to DI +/+ Non-Severe mice ($P<0.05$).

Table 3. Assumed papillary water contents, and calculated papillary tip values and cortico-papillary gradients (b) for osmolality, sodium, and urea in control mice and mice with ADH-resistant urinary concentrating defects

Type	Assumed papillary water content %	Osmolality		Sodium		Urea	
		tip *mOsm/kg TW* [a]	b *mOsm/kg TW per mm*	tip *mEq/kg TW*	b *mEq/kg TW per mm*	tip *mmoles/kg TW*	*mmoles/kg TW per mm*
VII +/+ (Control)	80	2771 ±246 [b]	426 ±45	741 ±66	118 ±12	1109 ±116	199 ±21
VII Os/+	82	2065 ±312	382 ±76 [d]	658 ±100	133 ±24	622 ±119 [c, d]	140 ±29
DI +/+ Non-Severe	85	1561 ±114 [c]	235 ±23 [c]	500 ±40 [c]	85 ±8 [c]	476 ±45 [c]	95 ±9 [c]
DI +/+ Severe	90	740 ±85 [c, d]	66 ±17 [c, d]	336 ±37 [c, d]	49 ±7 [c, d]	66 ±7 [c, d]	12 ±1 [c, d]
DI OS/+	87	911 ±80 [c, d]	114 ±19 [c, d]	336 ±32 [c, d]	56 ±8 [c, d]	101 ±16 [c, d]	19 ±4 [c, d]

[a] TW = Tissue Water.
[b] Mean ± SEM.
[c] Significantly different when compared to VII +/+ mice ($P<0.05$).
[d] Significantly different when compared to DI +/+ Non-Severe mice ($P<0.05$).

Results

The urinary osmolalities listed in Table 1 are similar to those previously reported for the various genotypes (3). Mice of the DI +/+ stock having severe diabetes insipidus have been mentioned previously [1–3] but were not emphasized. As is shown in Table 1, these mice (DI +/+ Severe) manifest a more marked concentrating defect than any of the other types; they excrete a hypotonic urine in a daily volume in excess of 150% of body weight.

The dimensional data are summarized in Table 2. As had been shown previously [3, 5], in both the VII and DI strains the Os gene is associated with a significant reduction in the length of the countercurrent system, i.e., in the distance from papillary tip to cortico-medullary junction. The slightly shorter countercurrent system (h_m) in DI +/+ Non-Severe and DI +/+ Severe mice, as compared to VII +/+ animals, may represent a strain difference.

The assumed papillary water contents, the estimated papillary tip values, and the cortico-papillary

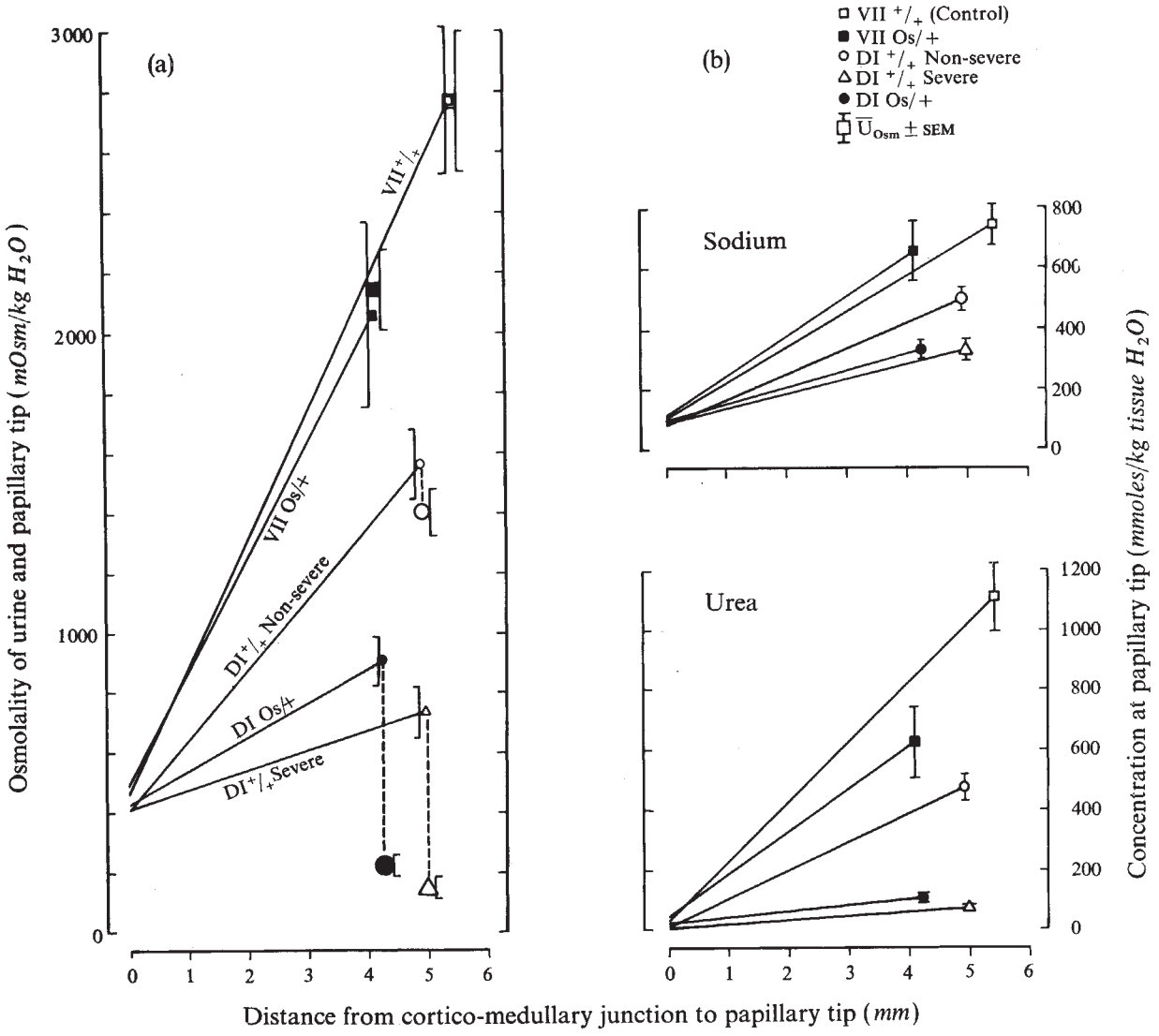


Fig. 2. a. *Urine osmolalities and estimated osmolalities at the papillary tip, plotted against the length of the countercurrent system in each group of mice.* The small symbols denote the papillary tip values; the large symbols, the urinary values. Each bracketed symbol represents the mean ± SEM. The difference between the mean papillary and urinary osmolality was not statistically significant for VII+/+, VII Os/+, and DI +/+ Non-Severe mice, and had P values < 0.001 for DI +/+ Severe and DI Os/+ animals. **b.** *Estimated sodium and urea concentrations at the papillary tip plotted against the length of the countercurrent system in each group of mice.* Each bracketed symbol denotes the mean ± SEM. Statistical analyses of the difference in length of the countercurrent system, in papillary tip values, and in the cortico-papillary slope among the various groups have been listed in Tables 2 and 3.

gradients for total osmolality, sodium and urea have been summarized in Table 3.

In Fig. 2a the mean urine osmolalities for each group listed in Table 1 have been compared with the estimated tissue osmolalities at the papillary tips. These values have been plotted against the length of the countercurrent system (h_m in Table 2). On the assumption that the cortico-papillary tissue osmolal gradient is linear [10, 11, 13], the slope of each line thus expresses this gradient (b in Table 3). The slopes and papillary tip values for sodium and urea have been graphed in a similar plot (Fig. 2b).

Control mice of the VII strain (VII +/+) have an osmolal gradient of 426 mOsm/kg tissue H_2O per mm. Their extrapolated mean papillary tip osmolality and mean urine osmolality are practically identical; this presumably reflects near or total osmotic equilibration between collecting duct fluid and papillary interstitium.

The effect of the Os gene in reducing renal size [3, 5] is reflected in the foreshortened countercurrent system, 4.11 mm in VII Os/+ as compared to 5.41 mm in mice of the same strain without the Os gene (Table 2 and Fig. 2). The cortico-papillary gradient for sodium is virtually the same in VII Os/+ as in VII +/+ mice, and the gradient for urea is mildly but not significantly reduced (Table 3 and Fig. 2). Consequently, the total osmolal gradient of 382 mOsm/kg tissue H_2O per mm in VII Os/+ animals is slightly but not significantly less than the observed value of 426 mOsm/kg tissue water per mm in VII +/+ mice. However, since the lesser gradients for both urea and total solutes are sustained over a shorter distance, their concentrations at the papillary tip are less in VII Os/+ than in VII +/+ mice. Again, the fact that the urine osmolality in VII Os/+ animals is not significantly different from the papillary tip osmolality presumably reflects sufficient water permeability to have resulted in osmotic equilibration between collecting duct and interstitial fluid.

Animals of the DI strain, whether they have severe diabetes insipidus (DI +/+ Severe) or only a mild concentrating defect (DI +/+ Non-Severe), have a countercurrent system which is only slightly shorter than that of control, VII +/+ mice (Table 2 and Fig. 2). Nevertheless, the tissue osmolality at their papillary tip is much lower than that which would be predicted from foreshortening of the countercurrent system alone. In both groups, there is diminution of the cortico-papillary gradient for sodium as well as for urea, and therefore of the total osmolal gradient. In DI +/+ Severe mice the osmolality of the urine is significantly lower than that at the papillary tip ($P<0.001$); this severe osmotic disequilibrium probably reflects deficient water permeability of the distal tubules and collecting ducts. The disequilibrium is very small and not statistically significant in DI +/+ Non-Severe animals ($P>0.2$); it therefore remains an open question whether a deficiency of water permeability exists in this group.

The Os gene appears to have about the same effect on shortening the countercurrent system when it occurs in the VII strain, VII Os/+, as in the DI strain, DI Os/+ (Table 2 and Fig. 2). Yet its occurrence in animals of the DI strain diminishes the cortico-papillary gradient for sodium, whereas no effect of this gene on the sodium gradient is discernible in VII Os/+ mice (Table 3 and Fig. 2b).[1] In contrast, a decrease in the urea gradient occurred in association with the Os gene in both the VII and DI, strains with the change being much more striking and statistically significant only in the latter group. Again, the highly significant difference between urine and papillary tip osmolality in DI Os/+ mice ($P<0.001$) probably reflects a defect in water permeability of the distal nephron.

Discussion

Estimation of the solute concentrations at the papillary tip involves three assumptions which need justification: a) that the cortico-papillary gradients for sodium, urea, and total solutes are linear; b) that papillary water content increases from 80% to 90% with increasing severity of diuresis; and c) that the shape of the mouse papilla resembles that of a pyramid or cone. Approximate linearity of the cortico-papillary gradient for total solutes was demonstrated in the original study of Wirz, Hargitay, and Kuhn [13], and confirmed by Ullrich and Jarausch [12] in various states of diuresis. The latter also depicted gradients for individual solutes, such as sodium, chloride, and urea in different diuretic states, as did Ruiz-Guiñazú, Arrizurieta, and Yelinek [10]. Although none of these studies showed a truly linear gradient, the data do suggest that linearity is a better assumption for the present estimations than the exponential function one might expect on theoretical grounds [14]. The discrepancy between the theoretical curve and measured experimental values is not surprising, since the theo-

[1] DI +/+ siblings of DI Os/+ mice resemble DI +/+ Non-Severe animals more than the DI +/+ Severe form.

retical calculations were based on only two apposing channels, reaching all the way to the bend of the countercurrent loop, whereas the biological reality is very different. In mice and most other species, the majority of countercurrent loops do not reach to the tip, and a third system of channels, the collecting ducts, yields water to the medullary region which tends to wash out the solute particles as it is removed by a fourth system, the vasa recta. In fact, a theoretical treatment of the entire system [15] shows gradients which are rather close to linearity for sodium, urea, and osmolality. At least one report [16] shows the sodium concentration to be higher in the middle of the medulla than in the outer medulla or papilla of dogs during the special circumstance of mannitol diuresis. This was not true, however, in other studies which examined different types of osmotic diuresis [12], nor was anything observed that even approached an exponential function. In summary, in assuming a linear gradient, we are saying only that available experimental data are more compatible with the existence of linearity than with some other simple function, not that they show true linearity.

Papillary water content has usually been found to be about 80% in antidiuresis [6, 11], and to rise to 90% in water diuresis [6] and osmotic diuresis [10, 11]. Although we did not determine the water content specifically in mice, there is no reason *a priori* to expect a different pattern. Furthermore, even if a single value of 80% were assumed for the water content of all groups, there would be no fundamental change in the data nor in their interpretation. The assumption of a pyramidal or conical shape for the mouse papilla may be slightly inaccurate since the papillary tip is sometimes blunted rather than pointed.[2] Although this would introduce an overestimate into our extrapolated values, it seems unlikely that a major error is involved.

Since the calculated osmolality at the papillary tip of control mice (VII +/+) concentrating their urine to nearly 3,000 mOsm/kg H_2O fell well within the statistical deviation of the urinary value, any systematic error in calculating the correction factor was probably less than 14%, and may in fact have been nil. Even though our method of extrapolation yields only an approximation, these admittedly rough estimates permit a much more useful interpretation than if the raw data in Tables 1 and 2 are considered alone.

The data shown in Figs. 2a and b provide some clues to the mechanisms leading to the concentrating defects in the various groups of mice. VII Os/+ mice appear to have high water permeability of the distal nephron, thus permitting osmotic equilibration between collecting duct fluid and papillary interstitium. Their deficiency apparently lies in building up the osmolal concentration at the papillary tip.

These mice are in renal failure and manifest an osmotic diuresis per nephron [3–5]. Although their total glomerular filtration rate is decreased, the filtered load of sodium per nephron is increased [4]; consequently more sodium than normal may be delivered to each loop of Henle and be largely reabsorbed by it [18]. This process may slightly overcompensate for the medullary washout effect of an osmotic diuresis, so that the cortico-papillary sodium gradient is slightly, although not significantly greater in VII Os/+ than in VII +/+ mice. Because this gradient is maintained over a shorter distance, the sodium concentration at the papillary tip is slightly but not significantly lower than normal (Table 3 and Fig. 2b).

The changes for urea are more striking, probably because of the known effects of an osmotic diuresis to decrease the fraction of filtered urea which is reabsorbed from the collecting ducts [19, 20]. This reduction in the medullary source of urea, coupled with a foreshortened countercurrent system, results in a urea concentration at the papillary tip which is significantly less than in VII +/+ mice (Table 3 and Fig. 2b). Thus the essential difference between the behavior of sodium and urea in the countercurrent system of VII Os/+ mice may be that the osmotic diuresis per nephron diminishes the medullary source of urea but not of sodium.[3]

In DI +/+ Severe animals, the large osmotic disequilibration between urine and papillary tip (Fig. 2a) probably reflects a deficiency in water permeability of the distal tubules and collecting ducts. It is possible that this defect can account not only for the hypotonic urine but also for the observed reduction in medullary solute concentrations. The suggestion that the absolute amount of water which is reabsorbed from the

[2] We decided to assume a pyramidal shape after first trying extrapolation with several other geometric forms. It is of interest that Aukland [17] also assumed a pyramidal shape and a linear gradient in calculating the medullary urea content of dogs.

[3] We had suggested earlier [3] that osmotic diuresis per nephron might not contribute importantly to the deficient urinary concentration associated with the Os gene. Subsequent experimental evidence by Stewart [4] as well as the present work has led us to change this view.

late collecting ducts may actually be greater in water diuresis than in antidiuresis [21, 22] has recently been supported by experimental data [23]. Insofar as in the steady state, this effect will obligate an increased egress of water and sodium out of the inner medulla, it will lead to a decreased cortico-papillary sodium gradient, even though the delivery of sodium to this area via the loops of Henle may be normal. Again, for urea the changes are much more striking because the medullary source for urea, i.e., reabsorption from the collecting ducts, is greatly diminished or abolished. Computer simulation of the countercurrent system [15] supports these explanations by showing that merely decreasing the water permeability of the distal convolution and collecting ducts will lead to changes in medullary solute concentrations quantitatively similar to those seen in DI +/+ Severe mice.

The small difference of 155 mOsm/kg H_2O between the urinary and papillary tip osmolalities in DI +/+ Non-Severe mice is statistically insignificant ($P > 0.2$). Nevertheless, if it is of biological significance, it might still reflect a minor deficiency in water permeability, and mechanisms qualitatively similar to those outlined in the preceding paragraph could then explain both the urinary and tissue changes in this group (the possibility of a minor defect in water permeability was not excluded by our previous studies [3] which showed that DI +/+ Non-Severe animals responded to ADH only if first given a water load, but not when drinking *ad lib;* i.e., the concentrating defect in these mice could not be corrected by exogenous ADH). On the other hand, the present data in these mice are equally compatible with the view that there is no deficiency of water permeability, and that the primary disorder involves a lack of buildup of the papillary osmolality. Methods other than those used in the present study will have to be applied to discriminate between these possibilities.

It is clear from Fig. 2a and b that the Os gene has a different effect when it is introduced into the VII strain than into the DI +/+ Non-Severe strain (see footnote 1). In the former, the Os gene decreases the cortico-papillary gradient for urea only, whereas in the latter it decreases the gradient not only for urea but also for sodium. It is possible that the combination of osmotic diuresis per nephron which is associated with the Os gene [3–5], plus a possible defect in water permeability which may be inherent to the DI strain, can account for all the deficiencies. Recent studies by Stewart [4], however, have revealed an additional abnormality. The introduction of the Os gene into the DI strain leads to severe shortening of the short loops of Henle, which in a segregating backcross generation, can be correlated with the severity of the urinary concentrating defect. No such shortening was observed when the Os gene occurred in the VII stock. Curtailment of the short loops of Henle might lead to decreased countercurrent multiplication for sodium in the outer medulla. This would diminish water reabsorption from cortical collecting ducts which, in turn, would decrease the papillary source of urea by reducing urea reabsorption from late collecting ducts. Again, only further studies can tell whether the large osmotic disequilibrium between urine and papillary tip reflects a severe deficiency in water permeability or whether the other abnormalities which have been identified in DI Os/+ mice might lead to this degree of osmotic disequilibrium in the face of a mild defect in water permeability.

As did those of Ullrich and Jarausch [12], these studies show the progressive and striking correlation between a defect in urinary concentrating ability and the decrease in the corticopapillary gradient for urea (Fig. 2b). The gradient for sodium is decreased much less, and the probable reason is that, while the washout due to water reabsorbed from the collecting ducts is common to both substances, the medullary source of urea but not that of sodium, is concomitantly diminished or obliterated. The present studies suggest that this selective decrease in medullary urea may be common to all diuretic states, whether they be due to increased osmotic flow, decreased water permeability, or a combination of these or other mechanisms.

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